Conclusion: this study suggests that synovitis is a common feature in painful knee osteoarthritis, associated with more severe chondropathy, but it also suggests that synovitis could be considered as a predictive factor of subsequent chondrolysis.

PP6

MACROPHAGES - ACTIVATED BY TGF-β PROMOTE OSTEOPHYTE FORMATION

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Purpose: Osteophytes (new formation of cartilage and bone) are important features of osteoarthritis. Growth factors like TGF-β have been shown to induce osteophyte formation through outgrowth of periosteal cells. Macrophages are important producers of a range of growth factors and as they cover the inside of diarthrodial joints, they may well form an important source of growth factors involved in osteophyte formation. In the present study, we investigated whether macrophages have an intermediate role in osteophyte formation.

Methods: In vitro, the interaction between murine macrophages and mesenchymal cells (precursors with chondrogenic potential) were studied using a Transwell system. Murine peritoneal macrophages, brought into the upper compartment, were cocultured with C3H10T1/2 mesenchymal cells present in the lower compartment. Spheroid neo cartilage formation was quantified under the microscope after staining with May Grunwald Giemsa. Various concentrations of TGF-β were added to macrophages. In vivo. synovial lining macrophages were selectively depleted by injection ol clodronate-laden liposomes prior to triple injections of 20 ng of TGF-b at alternate days. Total knee joint sections were taken at day 7 after the last injection and stained with safranin-O.

Results: Clustering and spheroid formation of C3H10Tl/2 was induced by TGF-β concentrations above 1 ng/ml. Lower concentrations (0.75 and 0.5 ng/ml) were ineffective. However, in the Transwell system, in the presence of macrophages, *0.5* ng/ml TGF-β was very effective in generating large spheroids, suggestive of macrophage-derived (co)factors. Using a specific ELISA, we found that in the co-culture supernatants, TGF-β concentration were lower in the presence of macrophages, making autoinduction of TGF-β unlikely and pointing to generation of other growth factors involved in spheroid formation. In addition, the contribution of macrophages to osteophyte formation was studied in vivo. Triple injections of 20 ng TGF-β into normal murine knee joints showed that at day 7 after the last injection significant osteophyte formation was observed at the lateral and medial site of patella and femur. Strikingly, removal of synovial lining macrophages prior to triple injection of TGF-β resulted in a drastic reduction (decrease - 80%) of osteophyte formation.

CONCLUSION: This study suggests that macrophages are crucial intermediates in osteophyte formation induced by TGF-β and apart from TGF-β, other macrophage derived (growth) factors promote this process.

PP7

A NOVEL PROTEIN-BINDING SITE (AGRE) IN HUMAN COLLAGENASE-3 PROXIMAL PROMOTER REGION IS INVOLVED IN REPRESSION OF TRANSCRIPTION

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Activation of collagenase-3 (coll-3) transcription in osteoarthritic (OA) cartilage involves proteins binding at sites such as AP- 1 and PEA-3. In this study, we have identified a novel protein-binding site, AAAAGAAAAAG (bp -117 to -127 in the coll-3 promoter), consisting of two copies of the pentanucleotide AAAAG separated by one nucleotide. This site was designated AGRE (AG-Rich Element). We demonstrate, for the first time that this site binds proteins that repress basal coll-3 transcription.

Method and Results: Human OA chondrocytes as well as four cell lines (COS-7, HEp-2 SW1353 and HeLa) were transfected with a plasmid consisting of the first 133 bp of the coll-3 promoter (containing the TATA box, the AP-1, PEA-3 and AGRE sites), and its AGRE mutated or deleted derivatives. Data revealed that the absence of a functional AGRE site, following its mutation or deletion, resulted in a significant increase in the coll-3 basal transcription in OA chondrocytes (183%, $p<0.02$; 221%, $p<0.03$, respectively). Deletion of the AGRE site also showed a 2-fold increase in the cell lines. No effect was found when chondrocytes were treated with coll-3 inducers, IL-1b and TGF-b. Two specific protein-AGRE binding complexes were detected by EMSA: a slower (complex #1) and a faster (complex #2) migrating complex. Their appearance seems to depend on the physiological state of the cell. Indeed, normal chondrocytes, synovial fibroblasts and the four cell lines showed only the complex #1. In OA chondrocytes, the complex number appearance discriminates two subgroups: the low-OA chondrocytes, showing low coll-3 basal levels and high inducibility of IL-lb stimulation (complex #1), and the high-OA chondrocytes, with high coil-3 basal levels and low IL-1b inducibility (complex #2). UV cross-linking revealed the presence of two major proteins with different molecular weight in complexes #1 and #2: 35 and 70 kDa for complex #1, and *45* and 100 kDa for complex #2. As above, the protein abundance was not influenced by either IL-1b or TGF-b.

Conclusion: We report a novel negative regulatory element in the coll-3 promoter. The presence of different protein-binding complexes is affected by the metabolic state of the cells. These findings suggest that the AGRE site plays a rate limiting step in coll-3 production, and may represent a likely target in the OA pathophysiology process.

PP8

COMPARISON OF HOMEOSTASIS OF THE EXTRA-CELLULAR MATRIX BY PHENOTYPICALLY STABLE CULTURED HUMAN CHONDROCYTES FROM NORMAL AND OSTEOARTHRITIC CARTILAGE OF THE SAME KNEE

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Objective: Synthesis and accumulation of the ECM is regulated by locally produced growth factors such as IGF and TGF-β. Turnover and degradation of the matrix is dependent on the responsiveness of the chondrocyte to catabolic cytokines such as IL-1α/β and TNF. The function of auto/paracnne anabolic (IGF-2/IGF-Re2) and of catabolic (IL-lα and B/IL-1 -Re) pathways in the homeostasis of the extracellular matrix (ECM) of nonnal and osteoarthritic (OA) articutar cartilage cells was investigated.

Methods: Phenotypically stable human articular cartilage cells were obtained from normal and OA cartilage of the same knee and maintained in culture in alginate beads over 1 week to reach